RESEARCH ARTICLE

Teaching microbial analysis techniques for the characterisation of baker's yeast through the inquiry-based laboratory

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Abstract: Laboratory course activities often focus on "cookbook style," so their activities have not yet integrated with scientific thinking as an essential aspect. In this research, the laboratory experience of microbial analysis techniques was redesigned using alternative inquiry to involve students in isolating and characterising yeast to make the bread rise. This research aimed to determine the effect of implementing inquiry-based laboratory experiences on improving students' technical skills and the structure and characteristics of laboratory courses. The method used a single case study to obtain a complete picture regarding implementing laboratory course redesign. Laboratory experience activities consisted of pre-laboratory (reinforcement of theory) and investigation (preparation of methods, laboratory investigation activities, data analysis and presentation, and writing written reports). Overall, our results suggest that inquiry-based laboratory experiences can improve students' microbial analysis technique skills in strong collaboration. In addition, students considered several benefits from this learning, such as developing designs and conducting laboratory investigations close to the original context. With these results, educators can use the redesign of this laboratory course without applying full "course-based undergraduate research experiences" to students.

Keywords: collaborative problem solving; inquiry laboratory; microbiology learning

Introduction

Laboratory activities in undergraduate programs with a "cookbook style" have been widely used (Rokos & Zavodska, 2020; Yusiran et al., 2019). Traditional approaches can be relied upon to demonstrate critical scientific principles and teach technical skills (Rokos & Zavodska, 2020; Wästberg et al., 2019), but traditional approaches have not yet integrated enough to encourage students to fully participate in the same investigative process as actual scientific investigative conditions (Madhuri et al., 2012; Yusiran et al., 2019). Student failure in designing experiments or observations is also associated with traditional laboratory teaching using manual cookbooks, which are still used on campus (Fadaei 2022; Zeidan & Jayosi, 2014).

Historically, laboratory teaching experiences in previous years have been courses that aimed to expose students to knowing methods and data collection without engaging them directly to develop hypotheses, design methods, or analyse data. In fact, the scientific process can encourage students to find solutions with a combination of knowledge or new analysis techniques (Huang et al., 2017; Solé-Llussà et al., 2022). To strengthen the structure and characteristics of practicum courses, we redesigned the laboratory experience focused on investigation. Inquiry-based learning (IBL) is integrated with laboratory investigation activities where students and lecturers act as learning partners (Charteris, 2019; Wen et al., 2023). IBL can provide ideal conditions for students to be actively involved in actual investigative activities, which scientists used (Kang, 2022; Peffer et al., 2015). IBL is also more effective than laboratory learning methods such as cookbooks and discovery approaches (Carriazo, 2011; de Jong et al., 2014).

In the context of this research, an inquiry-based laboratory experience was redesigned in the microbial analysis techniques course. The inquiry design used was alternative inquiry learning, which consists of procedural, conceptual and personal components (Bevins & Price, 2016; Kollöffel & de Jong, 2013). Students divided into five different investigations were conducted, each emphasising different sources. For example, the group identified yeast and studied their morphological and physiological characteristics using isolation and characterisation techniques. Alternative inquiry designs can provide more flexible approaches, leading them to act as scientists (Andersen & Garcia-Mila, 2017; Cirkony, 2023). The procedures were conducted appropriate to the recent research to find the most relevant answers (Darling-Hammond et al., 2020; Thomas, 2012).

On the other hand, alternative inquiry can be used as a fresh idea for lecturers so that students become collaborative and innovative learners (Cirkony, 2023; Ovens, 2011). Students also should be able to develop problem-solving abilities and build teams to solve recent complex problems (Bradforth et al., 2015; Darling-Hammond et al., 2020). Therefore, students worked together in groups consisting of four to five people. Each group was given a topic related to the isolation and characterisation of yeast that have the potential to make bread rise. We believe the inquiry process requires student communication and collaboration (Guez et al., 2017; Lewis et al., 2021).

Additionally, each group was directed to conduct a literature review regarding yeast and its potential to aid their understanding of its novelty and relevance. Then, they developed research questions and hypotheses to determine whether the identified yeast could make bread rise. In general, students need to gain direct experience in formulating research questions (Baur & Emden, 2021; Zhu, 2015), so a literature review should be conducted to guide them in correctly formulating research questions. In the previous study, the level of student skill acquisition in composing questions was relatively low (Chin & Osborne, 2008; Šmida et al., 2023), even though defining a research problem is the initial stage that should be conducted in the investigation process (Pedaste et al., 2015; Svanes & Andersson-Bakken, 2023).

The alternative inquiry design can encourage students to answer central questions about bread-baking yeast using several microbial analysis techniques. Students used the knowledge gained in pre-laboratory activities to form a comprehensive answer to the question, "Does the yeast obtained from fruit X have the potential to make bread rise?" By approaching the same question from various microbial analysis techniques, students can better understand the mechanisms to determine yeast to make bread rise. Therefore, the main goal of redesigning this laboratory activity was to increase students' involvement in the authentic inquiry process and help them develop scientific skills. To determine the extent to which the goal can be achieved, we measured students' acquisition of microbial analysis techniques skills, characteristics and structure of laboratory activities, and students' perceptions of the benefits of these activities. Our work intends to engage students in laboratory techniques in the field of microbiology that enable them to deal with microbial physiological concepts and, more precisely, to make learning relevant to the 21st century. This research applies inquiry pedagogy, with a focus on laboratory activities as an important foundation in the 21st century.

Method

Research Design

Case study research design can provide an understanding of real-life classroom situations (Sáez Bondía & Gracia, 2022). For this study, we conducted a case study research design in the microbial analysis techniques course at the Biology Study Program, Universitas Islam Negeri Maulana Malik Ibrahim Malang. We conducted the case study research through the following steps: The first author determined the sample, collected, and analysed data. Meanwhile, the second and third researchers analysed qualitative data (Hsu & Van Dyke, 2021). We chose this method based on previous research experience (Ncala, 2020), which aimed to explore students' active involvement and encourage interactive learning in inquiry learning.

Context and Participants

We collected data from the class, especially in the elective course on microbial analysis techniques conducted in the odd semester of 2022. The participants in the study were 24 students in their fourth year who had exposure to general microbiology and the basics of biology laboratories when they were in the first and second years. Thus, students who entered our course already have basic knowledge about microorganisms and their characteristics in terms of morphology and physiology aspects. Students who participated did not have a strong background in yeast to make the bread rise. During the pre-laboratory courses, lecturers taught the conceptual information and procedures used to isolate and test the potential of yeast to make the bread rise. Specifically, the discussion of the function and purpose of each technique was discussed during the pre-laboratory course. Besides conceptual knowledge, students also understood microbiology laboratory safety protocols about the aseptic technique. The aseptic technique should be used for all laboratory procedures when working with microorganisms (Aruscavage, 2013). However, teachers and teaching assistants should make students aware of work safety in the laboratory by using equipment such as gloves and laboratory coats.

Procedure

Because the students did not understand the isolation process and characterisation of the yeast used. Thus, we dedicated pre-laboratory courses to studying theories about isolating microorganisms from different sources, characteristics of microbial groups, control of microbial physical and chemical agents, and methods in microbiology (e.g. pure culture techniques, microbial nutrition). Pre-laboratory courses were implemented for four weeks, whereas inquiry-based laboratory experiences were implemented over ten weeks. We conducted different activities in the inquiry-based laboratory weeks, such as method preparation, investigation, data analysis, and written reports. In the first three weeks, students were preparing the method. Five weeks later, students were investigating and data analysis, so for the last two weeks, students were presenting the results as a scientific article.

The laboratory processes conducted by students were composing media, yeast isolation, purification, temperature tolerance test, ethanol tolerance test, 50% glucose tolerance test, and flocculation test. The media used were Yeast Malt Extract Agar (YMEA), Yeast Malt Broth (YMB), Glukosa Yeast Pepton Broth (GYPB), and Yeast extract Peptone Glucose Broth (YPGB) media. Isolation was conducted by taking 15 grams of snake fruit and placing it in 50 ml of YMB media, then incubating it for 48 hours. The incubation results were diluted to 10^{-3} and inoculated into YMEA media using the spread plate method. Next, the isolate was incubated for 72 hours. The grown yeast isolate was inoculated in 3 ml of YMB and shaken at room temperature for 48 hours. Finally, 20 µl of the isolate was taken and inoculated on YMEA media using the spread plate technique (incubation for 48 hours at 27°C). Results identification of the yeast isolates found from snake fruit showed that the yeast belonged to the Ascomycetes class. Table 1 summarises the test steps from the results in the laboratory processes.

During the investigation process, lecturers tried to encourage students to understand the function and purpose of each technique used so that they gained an understanding of answering questions prepared by various microbial analysis techniques (see Table 1). Therefore, this course has been designed considering three learning objectives. First, students demonstrate an understanding of theory, practice, and investigation design for isolation techniques and characterisation of yeast to make the bread rise. Second, students demonstrate the ability to formulate questions, develop hypotheses, and predict results. Third, students can summarise experiences and demonstrate their knowledge through written communication.

Table 1. Steps and results in the laboratory processes

Stages	Steps	Descriptions	Results
Stage 1	Temperature tolerance	1 ml of yeast isolates from YMB media were inoculated into 9 ml of YPGB (incubation for 72 hours) at three temperature conditions (25 ºC, 37 ºC, and 40ºC).	Yeast incubated at 37ºC had the highest density value, 2.926.
Stage 2	Ethanol tolerance	Yeast isolates from 48-hour YMB media were inoculated into YPGB media with varying ethanol concentrations (10%, 13%, and 15%).	Yeast incubated at 10% yeast ethanol concentration had the highest density value, 0.925.
Stage 3	Glucose tolerance 50%	Yeast isolates were inoculated into YPG media with a glucose concentration of 50% (m/v) and incubated at 25ºC for 48 hours.	The yeast density value was 1.011 (high).
Stage 4	Flocculation	Yeast isolates were inoculated in 10 ml of YPG broth and then incubated at 30ºC for 72 hours.	Formed sediment under the media and produced foam at the top of the media.

Data Collection and Analysis

We collected the data through two main instruments: assessment of microbial analysis technique skills and evaluation of the structure and characteristics of the class. The level of microbial analysis technique skills was assessed using a rubric developed from four indicators of science efficacy (Robnett et al., 2015), consisting of investigation design, data collection, data analysis and presentation, and scientific communication. We measured at the beginning and the end of each phase. Then, we converted the data from observation results into numerical data with a maximum value of 100. Next, the pre-test and post-test values were compared using a paired t-test to show student skills changes after participating in inquiry-based laboratory activities.

Meanwhile, the structure and characteristics of the class were also assessed to determine the extent to which the laboratory experience focused on scientific inquiry. The structure and characteristics of the class were evaluated using a rubric with three indicators (collaboration, discovery and relevance, and iteration) adapted from Laboratory Course Assessment Survey (LCAS) (Corwin et al., 2015). LCAS was not presented in questionnaire form because students would not understand the difference between course research experience and actual research experience, so only lecturers recognised this difference (Beck & Blumer, 2021). The observation results are converted into numerical data with a maximum value of 100. We measured LCAS throughout the semester, which was presented in average form.

To strengthen LCAS, we also asked students an open question: "What do you feel after joining this course?" Then, we analysed the qualitative results using content analysis techniques. The coding units were based on the importance of context in understanding student statements, and only the first keyword in a sentence was coded. Three people conducted the coding stage: the lead author and two others. To ensure reliability, the Cohen Kappa substantial agreement was confirmed to reach more than 0.75. Each code was presented in relative frequency form to show how often students mentioned each code.

Results and Discussion

We provide a laboratory course design to enhance laboratory activities' structure and inquiry characteristics. We used two primary assessments to evaluate whether this design impacted students' microbial analysis technique skills and laboratory class structure. In addition, we also assessed how students perceived their experience in the inquiry-based laboratory.

Laboratory Technique Skills

The increasing students' laboratory technique skills (pre-post) were examined using paired t-tests, and a significant increase was observed in all four categories (experimental design, data collection, data analysis and presentation, and science communication) with total $p < 0.01$ (Table 2). In line with Cheng et al. (2021), IBL can develop the ability to ask research questions, design experiments, and engage in problem-solving activities. All aspects were significantly increased because the inquiry represented a constructivist (Serafín et al., 2015). The teaching approach used in inquiry-based laboratory courses was student-centred, dynamic (Ernst et al., 2017), and focused on finding answers to research questions (Aditomo et al., 2013).

In general, this research has documented that implementing laboratory-based IBL allowed students to improve their technical skills and research processes (Barret, 2017; Strom, 2015). This research also filled the gap regarding the articulation of laboratory-based IBL processes more completely (Tsybulsky et al., 2020), where this research not only questions the practical aspects but also captures the meaning of each process that students follow (Prinsen, 2016). Demaria et al. (2019) pointed out that inquiry-based experiences in final-year students, similar to the population in our research, can facilitate them to investigate real-world problems rather than simply getting the expected answers. Our students can improve their experimental design skills, the importance of the aseptic technique, yeast isolation and culture steps, and a series of physiological tests to demonstrate the potential of yeast to make bread rise.

Table 2. The Improvement of students' laboratory technique skills

No	Aspect	Pretest (M ± SD)	Postest (M ± SD)	Mean Paired Difference	*t*	*p*
1	Design	82.79 ± 4.34	91.62 ± 2.84	-8.83	-11.542	0.000
2	Data collection	77.37 ± 6.05	94.58 ± 2.04	-17.20	-14.944	0.000
3	Data analysis	86.25 ± 8.24	91.83 ± 4.51	-5.58	-3.075	0.005
4	Communication	86.25± 6.80	92.50 ± 5.89	-6.25	-3.880	0.001
5	Total	332.67 ± 14.86	370.54 ± 8.03	-37.87	-12.650	0.000

The highest increase was in the data collection aspect (from 77.47 to 94.58), whereas the smallest increase was in the data analysis aspect (from 86.25 to 91.83). The data collection has increased due to students' success in identifying variables under investigation (Šmida et al., 2023) and formulating research questions while designing experiments (Gholam, 2019). If the students know the measured variables, they can control the experimental system or observation (Mohd Saat, 2004). Students were encouraged to collect relevant data to answer questions (Dobber et al., 2017; Ellwood & Abrams, 2018), but they were still challenging to analyse and conclude, even after taking this course. In line with the findings, students had a high average in experimental procedures but a low average in processing, analysing and interpreting the data (Šmida et al., 2023). If analysing and interpreting data had a low average, it can have implications for low scientific communication skills, as was found in our study. Students struggled to identify trends and significant data (Lati et al., 2012). Furthermore, students also seem to have big problems interpreting data in graphical form (Beaumont-Walters & Soyibo, 2001), where the ability to read and understand mathematical symbols and basic graphs is a critical element in scientific communication skills (Özden & Yenice, 2022). Thus, this condition requires further investigation on improving student performance regarding data representation in scientific communication.

LCAS Score

LCAS was used to assess the structure and characteristics of the laboratory that focused on inquiry, particularly in the areas of collaboration, iteration, discovery and relevance. Our findings showed that LCAS scores in microbial analysis techniques courses with inquiry-based laboratory activities were overall high for all categories. Overall (Figure 1), the collaboration category score was higher (M= 92.08; SD= 9.46) than the iteration category (M= 87.95; SD= 4.38) and discovery and relevance (M= 89.50; SD= 8.91), which showed that inquiry-based laboratory has very strong for building collaboration between students.

These findings support previous research that skills can be improved with collaboration and exchange of ideas (Hämäläinen & Vähäsantanen, 2011). The collaborative context in this research can facilitate students to build ideas with others to achieve the understanding needed to solve problems (Zhou, 2015). Barret (2017) showed that the main characteristic of IBL is students are actively involved in every process to build knowledge through dialogue activities with other people. Thus, laboratory activities with IBL can also be defined as collaborative group activities to solve meaningful problems (Yew & Goh, 2016).

In general, a high LCAS score showed that students could participate in laboratory activities like real research experiences (McLaughlin & MacFadden, 2014), thereby enabling students to engage with evidence-based phenomena (McNeill & Berland, 2017). Our findings also aligned with previous publications stating that students can experience a high level of "authenticity" in courses designed similarly to original investigations (Rowland et al., 2016). Students learn science process skills by actively engaging in inquiry-based laboratory experiences (Jeffery et al., 2016). Thus, this alternative inquiry can be used as a forum for students to bring their work together with the outcome of scientists (Sandika & Fitrihidajati, 2018).

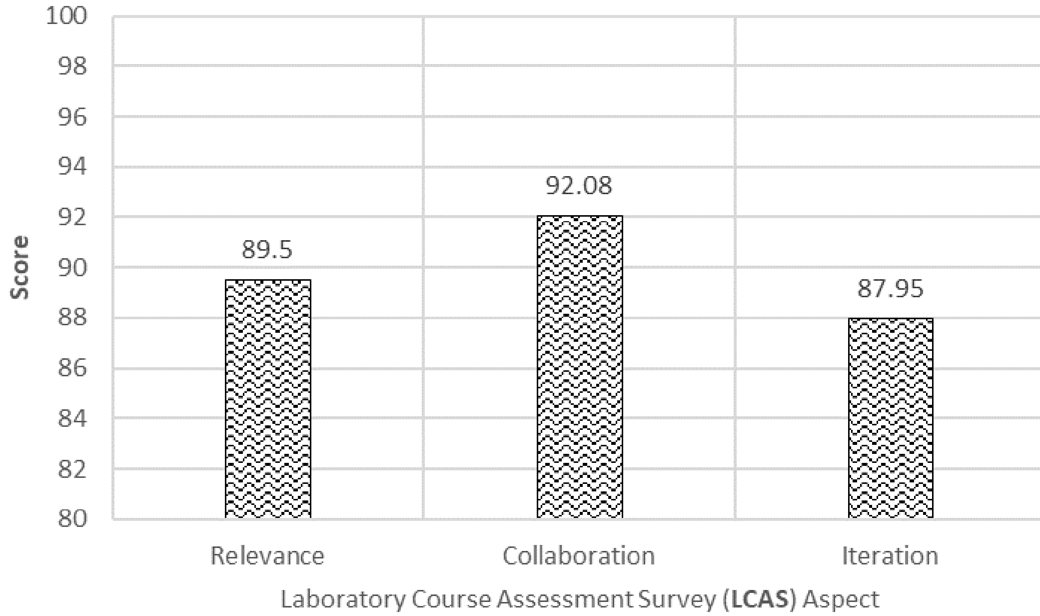

Figure 1. Student LCAS scores after participating in laboratory activities integrated with IBL

Qualitative Analysis of Student Perceptions

Qualitative data were analysed to explore student perceptions of the usefulness and relevance of inquiry-based laboratory research experiences. Students were more likely to comment on their level of interest in the laboratory, with more frequently reporting positive comments (88.46%) and fewer negative comments (11.54%). In general, almost three-quarters (76.92%) of students agreed that inquiry-based laboratory experience could help them to develop their abilities in designing and conducting investigations in the laboratory:

"The investigation is easier because we are involved in designing the method" S3.
"I easily remember what should be done during an investigation" S7
"Learning like this helps me to learn to design the right investigations" S14

More than half (54.17%) of students agreed that this course could provide accurate and actual research experience.

"I feel like I'm in the real research, not in practicum" S8
"This laboratory activity provides experience for conducting real research" S20
"From design to writing, this is the process that should be conducted as a scientist" S21

Meanwhile, less than a fourth of students (20.8%) stated that their scientific writing skills had improved.

"Report writing is different from usual, I learned a lot about writing" S4
"Writing articles is a challenge, but in this course, it seems easier" S9
"I learned what should be in a scientific article" S16

Despite most students being positively referred to IBL, only around three (11.54%) responded negatively to IBL. Around three students commented that data analysis techniques were still complicated even though they had participated in laboratory experience activities.

"Many microbial analyses have been conducted, but it is still difficult to determine" S2
"Processing and engagingly presenting results remains a challenge" S24
"Of the many processes, the analysis section needs to be improved further" S1

Our research examined the development of students' technique skills through IBL-based laboratory courses. Most students commented more positively that they had acquired these skills after taking courses with strong characteristics and structured collaboration. The finding showed that IBL-based laboratory experiences can create collaboration and meaningful learning processes (Rozenszayn & Ben-Zvi Assaraf, 2011) and create positive student feelings when conducting scientific investigations (Demaria et al., 2019). These findings also confirmed that based on students' perceptions of laboratory activities, collaborative work and inquiry can improve their technical microbial analysis skills. In other words, an intentional program focusing on IBL-based laboratory activities will help students be more aware of the effectiveness of inquiry-based methodologies (Connolly et al., 2022).

Students considered that an IBL-based laboratory can stimulate them to develop new knowledge and techniques and strengthen their ability to determine hypotheses and report them in scientific articles. Based on the students' perceptions, it can be concluded that an IBL-based laboratory positively encourages the transferability of critical inquiry into the context of more effective laboratory experiences (Munthe & Rogne, 2015). Understanding scientific inquiry in this laboratory program can involve knowledge of the inquiry process and the ability to conduct inquiry. Therefore, this research explains what students know about scientific inquiry and its impact on their skills (Gyllenpalm et al., 2022). These results reflect the literature that successfully implementing an IBL-based laboratory is very complex (Prinsen, 2016).

By mapping students' laboratory technique skills, we have provided a tool for educators to select what aspects of skills need attention in inquiry activities and identify future research avenues related to the development of students' laboratory skills. Based on the negative statement given by students, in the future, greater emphasis can be placed on developing students' analytical skills (Lee 2023), both in selecting methods for microbial physiological analysis and managing the results obtained (Pols et al., 2021). In addition, there needs to be an increase in students' ability to recognize ambiguity in analyzing and identifying yeasts that have the potential to make bread rise.

Conclusion

This research provides empirical evidence that we found positive student outcomes by strengthening them with inquiry-based laboratory experiences in a microbial analytical techniques course. Students demonstrated significant improvements in laboratory technique skills by maximising collaboration in one workgroup. Students also expressed their ability to design and investigate laboratory activities has increased.

Practical Implications

This research showed that the inquiry-based laboratory experience designed in the microbial analysis techniques course could encourage students to learn the main aspects of laboratory techniques and work collaboratively during the investigation process. Hence, lecturers can provide partial Course-based Undergraduate Research Experiences (CUREs) to help students gain critical scientific skills. In addition, lecturers can focus on students' understanding of their work's novelty and value.

Limitations and Suggestions for Future Research

Although this research showed promising results, several limitations need to be highlighted in this research. We realise that the student population in this research was conducted using a convenience sampling technique from one university, so it must represent something other than the broad undergraduate population. In addition, this data collection did not use a control group and was replicated in several semesters and different institutions. We recommend further research to conduct quasi-experiments by considering multiple demographic factors such as semester and gender to determine the broader significance of the inquiry-based laboratory experience offered in this study.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author Contributions

M. Saefi: methodology, analysis, writing original draft preparation, and review and editing. **W. C. Adi:** writing original draft preparation, and review and editing. **A. Rahmasiwi:** writing original draft preparation, and review and editing. **H. Maghfiroh:** writing original draft preparation, and review and editing. **M. E.**

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